APPENDIX A - Protocol

STILLMEADOW
INCORPORATED

PROTOCOL For STUDY 24072-20

Test Substance:	BEHR Antibacterial Paint, #3190
Study Title:	ACUTE INHALATION TOXICITY In RATS
Guideline:	OCSPP 870.1300
Test Facility:	STILLMEADOW, Inc. 12852 Park One Drive Sugar Land, TX 77478

Approved: _____ 04 Nov 20
Andrew Doig, MS — Date
Study Director, STILLMEADOW, Inc.

Approved: _____ 02.Nov.20
Management, STILLMEADOW, Inc. — Date

Reviewed: _____ 04 NOV 20
Kristina Rodrigue, RQAP-GLP — Date
Quality Assurance Director, STILLMEADOW, Inc.

Sponsor: BEHR Paint Company
1801 E. St. Andrew Place
Santa Ana, CA 92705
714 975 3127
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Approved: _____ 11/03/2020
John Gilbert — Date
Chief R&D Officer

A. GENERAL

1. Study Title:	Acute Inhalation Toxicity in Rats
2. Purpose:	To determine acute inhalation toxicity potential of test substance in rats.
3. Method Guidelines:	This study will be conducted according to US OCSPP 870.1300.
4. Regulatory Compliance:	This study will be conducted in compliance with Good Laboratory Practice (GLP) standards: 1. EPA FIFRA 40 CFR 160 In the event of a regulatory inspection, Regulatory Inspectors will be provided with all study documentation requested. Sponsor will be notified of inspection of their study. All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOP).
5. Quality Assurance:	The Quality Assurance Unit (QAU) will review the protocol. Study information will be entered into the master schedule. In-progress inspection(s) will be performed to ensure integrity of the study. Any deviations from SOP, protocol or GLP standards will be reported to Study Director and Management. Raw data and report will be audited, and a statement prepared and signed which will specify dates inspections were made and findings reported to Management and Study Director.
6. Test Substance:	BEHR Antibacterial Paint, #3190. Test substance identification should include name, lot/batch number and purity. Sponsor should also provide information regarding safety, storage conditions and disposal. Sponsor assumes responsibility for purity, stability, identity, synthesis methods and location of documentation.
7. Proposed Schedule:	Preliminary analyses or definitive portion should begin after test substance receipt, authorization to conduct study and study initiation. Proposed Experimental Start & End: 11 Nov 20 - 25 Nov 20 Study will be extended if extra dose levels are required.
8. Study Director:	Andrew Doig, MS
9. Experimental Summary:	Test substance will be administered for 4 hours in either a 15 L nose-only chamber, or 500 L stainless steel, dynamic flow, nose-only test chamber. Nominal, gravimetric, and/or analytical determinations of chamber concentration as well as particle size determinations will be made. Animals will be observed frequently on exposure day for mortality and pharmacologic and/or toxicologic signs, and once daily after for 14 days. Histopathology will be available at Sponsor request. If sufficient number of dose levels are tested, LC_{50} with slope and 95% confidence limits will be calculated.
10. Protocol Amendments:	Any protocol alteration will be justified, approved by Study Director, and recorded in writing.
11. Sponsor Audits:	Sponsor may send an authorized Representative to inspect test system and/or data on STILLMEADOW, Inc. premises during normal working hours.

B. EXPERIMENTAL DESIGN

1. Animals

a. Species/Strain/Source:	Albino rat / Sprague-Dawley / Texas Animal Specialties; Humble, TX (or other suitable supplier)

B. 1.	b.	Species Justification:	The rat is conventionally used to provide an index of toxicity on which human hazard can be judged, and is preferred by regulatory agencies.
	c.	Quantity & Sex:	5 male, 5 female (nulliparous & non-pregnant) for initial dose level & 5/sex for any additional dose levels, as required (see B.3.f.)
	d.	Age/Weights on Day 0:	8 - 12 week / Male: 225 - 375 g, Female: 175 - 250 g Weight variation should not exceed ± 20% of mean for each sex
	e.	Identification:	Ear punch
	f.	Acclimation & Health Status:	Animals will be acclimated for at least 5 days prior to exposure. Normal weight gain, appearance and behavior will be factors used to select healthy naive animals for testing.

2. Animal Husbandry

a.	Cage Type:	Polycarbonate box with bedding
b.	No./Cage:	Housed individually during observation period
c.	Enrichment:	Provided to each animal during study
d.	Food:	Teklad Global Diets® #2018, or equivalent, available ad libitum prior to & after exposure; analyzed by manufacturer for nutritional content
e.	Water:	Tap water (available ad libitum prior to & after exposure), automatic system; municipal water supply analyzed by TCEQ Water Utilities Division
f.	Contaminants:	There are no known contaminants in feed or water available to laboratory animals that would be expected to interfere with this study.
g.	Room Environment:	Target temperature: 22° ± 2°C Target relative humidity: 30 - 70% 12-hr light/12-hr dark cycle (regulated automatically) Ventilation: at least 10 air changes per hour

3. Test Substance Administration

a.	Reason for Route of Administration:	Inhalation is a potential route of human exposure.
b.	Test Chamber/Housing:	Test substance atmosphere will be established inside either a 15 L nose-only chamber or 500 L stainless steel, dynamic flow nose-only test chamber (animals individually housed in polycarbonate tubes).
c.	Preliminary Analysis:	A variety of techniques will be used in attempt to attain a **limit concentration of 2 mg/L** test substance in the chamber while also attempting to obtain a particle size distribution with mass median aerodynamic diameter (MMAD) of 1 - 4 microns. If particle size generated is too large and/or a suitable concentration is not attained, Sponsor will be notified. Similar techniques will be used for extra concentrations (if any) required/requested. For gaseous test substances, particle size will not be assayed.
d.	Dosing:	Test substance will be administered as either aerosol or gas in the test chamber. Filtered air will be used for dilution.
e.	Exposure Duration:	Four hours after equilibration of chamber conditions
f.	No. of Animals & Dose Level Selection:	Ten rats (5/sex) will be exposed for 4 hours to optimum limit concentration determined by pre-exposure testing and/or Sponsor request. If LC_{50} is greater than the limit test level, no further testing is necessary. If mortality exceeds 40% in either or both sexes, then an additional concentration of ~0.5 mg/L (or alternate lower-limit exposure concentration selected by Sponsor) of test substance will be tested for LC_{50} for appropriate sex.

B. 3. f. (cont.) There will be at least five animals (5 male and/or 5 female) per exposure level. If both sexes are tested at a given exposure level, the group will contain equal numbers of males and females. No formal randomization procedure will be required.

g. Control Groups: At Sponsor request, a sham, vehicle or negative control group can be run concurrently.

h. Operating Parameters: Operating parameters to be measured include the following.
Airflow: Monitored through use of calibrated orifice plate & sufficient to insure adequate oxygen content (at least 19%) of exposure atmosphere. Airflow will equal at least 12 air changes per hour.

t-99: Calculated for each exposure & depends on air flow. Start time of exposure will be adjusted accordingly.

Temperature & humidity: Measurements will be taken at 30-minute intervals during exposure period. Chamber targets are 22° ± 2°C & 30 - 70% RH.

Analytical concentration determination: When applicable, at least hourly for each exposure concentration, using procedures supplied by Sponsor or developed by STILLMEADOW, Inc.

Gravimetric concentration determination: When applicable, once to twice/hour.

Nominal concentration: Determined once for each exposure.

Particle size: Determined at least twice with cascade impactor. Results reported will include MMAD size & geometric standard deviation (aerosols only).

4. Observations

a. Clinical Signs: Observations for mortality and pharmacologic and/or toxicologic signs will be made frequently on the day of exposure and once daily after for 14 days. Nature, onset, severity and duration of all gross or visible pharmacologic/ toxicologic signs will be recorded. Observations will include: skin, fur, eyes and mucous membranes, somatomotor activity and behavior pattern; particular attention will be given to tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Animals with significant signs of pain/distress will be given a sufficient concentration of suitable analgesic by subcutaneous or IM injection; analgesic will be readministered at appropriate frequency as needed.

b. Body Weights: Body weights will be recorded on Days 0 (prior to exposure), 7 and 14, or at time of discovery after death.

c. Animal Sacrifice: Animals with signs of severe pain/distress considered irreversible will be humanely euthanized, per Study Director decision. All animals surviving to termination will be euthanized by IP injection of Fatal Plus®.

d. Necropsy: Gross necropsy will be conducted on each animal at termination or time of discovery after death, and results recorded (generally only abnormalities if any, or NOA if none). Gross necropsy shall include gross observations of external surfaces; all orifices; and thoracic, abdominal and pelvic cavities. At Sponsor request, sections of abnormal tissues will be saved in 10% neutral buffered formalin for possible histopath examination; tissues will be discarded if histopathology is not performed.

5. Evaluation of Results: Unless only a single limit exposure concentration is tested, LC_{50} with (if applicable) slope and 95% confidence limits will be calculated for males, females, and sexes combined, by method of Rosiello, Essignmann and Wogan: Rapid and Accurate Determination of Median Lethal Dose and its Error with a Small Computer, Journal Toxic Environ Health, 797 - 809, 1977, or other appropriate method. Toxicity Category may be assigned from LC_{50}.

B. 6. Test Substance Accountability:

A comprehensive inventory of test substance received and used will be kept. Test substance container(s) will be weighed when received at this facility, and all test substance use recorded. Test substance and substance dosing solutions will be stored in original containers or equivalent, or in capped glass containers.

7. Unused Test Substance Disposal:

Unused test substance will be disposed of at Sponsor's expense after study termination.

8. Safety Precautions:

General safety precautions required by laboratory SOP will be followed. Sponsor will supply basic toxicity data on test substance to be used; however, since toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. Sponsor or Representative shall be notified of any exposure requiring physician's exam or care.

C. DATA MANAGEMENT

1. Records:

The following records will be maintained at STILLMEADOW, Inc. during the study, and archived upon study termination:

a. Protocol & protocol amendments (if any)
b. Final report & amendments (if any)
c. Study correspondence
d. Animal receipt/acclimation data
e. Test substance receipt, identification supplied by Sponsor, administration, disposition
f. Test animal information: number, species, age, sex, source, strain
g. Body weight data
h. Daily observation data for pharmacologic &/or toxicologic signs
i. Mortality data & gross necropsy findings, & histopath data if requested
j. LC_{50} & calculations (if any) of slope with 95% confidence limits
k. Chamber operating parameters
l. Other pertinent data

2. **Data Storage**:

All raw data, originals of protocol, final report, any amendment(s) and a test substance sample will be archived at STILLMEADOW, Inc. for 15 years.

3. Data Reporting:

Final report will include following data as described in GLP standards:

a. Statement from QAU
b. GLP Compliance Statement & signature of Study Director
c. Names of scientific personnel involved in study
d. Dates of study initiation & termination
e. Identification, label information, description, preparation, storage of test substance
f. All pertinent animal data & husbandry, exposure information, operating parameters, observation methods
g. Description of test procedures
h. LC_{50} &, if calculated, slope with 95% confidence limits for males, females, & sexes combined
i. Individual body weights
j. Observations on nature, onset, severity & duration of all gross or visible pharmacologic &/or toxicologic signs; nonroutine findings will be addressed in a discussion section
k. Individual mortality data, gross necropsy findings, & histopath findings if any
l. Copy of this protocol; deviations (if any) & impact on study

4. Report Submission:

A final report will be generated after termination of in-life portion of the study (and histopath, if any, completion); a draft report may first be issued for Sponsor approval.